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| (21) International Application Number: PCT/AU99/00087 (22) International Filing Date: 15 February 1999 (15.02.99) (30) Priority Data: PP 1823 13 February 1998 (13.02.98) AU (71) Applicant (for all designated States except US): GARVAN INSTITUTE OF MEDICAL RESEARCH [AU/AU]; St Vincent's Hospital, 384 Victoria Street, Darlinghurst, NSW 2010 (AU). (72) Inventors; and (75) Inventors/Applicants (for US only): SCOTT, Kieran, Francis [NZ/AU]; Unit 3/4 Kent Street, Waverley, NSW 2024 (AU). BRYANT, Katherine, Jane [NZ/AU]; 3/79 Smith Avenue, Allambie, NSW 2100 (AU). CHURCH, Bret, William [AU/AU]; 17 Yowie Avenue, Caringbah, NSW 2229 (AU). INGLIS, Adam [AU/AU]; 39 Buena Vista Drive, Rye, VIC 3941 (AU). TSENG, Albert, Peng, Sheng [AU/AU]; 6 Wyvern Street, Epping, NSW 2121 (AU). (74) Agent: F.B. RICE & CO.; 605 Darling Street, Balmain, NSW 2041 (AU). | (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> | |
| (54) Title: CYCLIC PEPTIDE INHIBITORS OF PLA2 (57) Abstract The present invention provides cyclic peptides and compounds which inhibit the enzyme activity of phospholipase A2. The preferred compounds are cFLSYK, cFLSYR and c(2Nap)LS(2Nap)R. | | |

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Cyclic peptide inhibitors of PLA2

Field of the Invention

The present invention relates to cyclic peptide inhibitors of PLA2. In addition, the present invention relates to pharmaceutical compositions including as the active ingredients these inhibitors, and to methods of treatment involving administration of these inhibitors.

Background of the Invention

Secretory phospholipases A2 (sPLA2s) are a family of calcium dependent 14kD enzymes, that catalyse the hydrolysis of the sn-2 fatty acyl ester bond of phospholipids (1). These enzymes, which were first described as components of snake venoms and later in mammals, are classified into four major classes, Type I, Type II, Type V and Type X based on their primary structures (42). Type I PLA2 of mammalian origin is mainly found in the pancreas (2) while the Type II enzyme is stored in secretory granules in blood platelets, macrophage and neutrophils (3, 4) and in tissues, is localised in mast cells, paneth cells and chondrocytes (5, 6). It is also found in fluids derived from patients with inflammatory conditions (7, 8) and is induced in several cell types in response to inflammatory stimuli (5). Type V and Type X PLA2s are less well characterized. Type II PLA2 has therefore been implicated in the pathogenesis of several inflammatory diseases in humans such as rheumatoid arthritis and septic shock (56,57). Murine, inhibitory monoclonal antibodies raised against synovial PLA2 have demonstrated pre-clinical efficacy. Accordingly, there is interest in the development of compositions which inhibit the enzymatic activity of PLA2.

Despite differences in their primary sequences (approx. 30% homology only), crystal structures of PLA2s from bovine pancreas (Type 1) and human synovial fluid (Type II) are almost superimposable and, not surprisingly, the active sites from both types of enzymes are virtually identical (9). Asp 99 and His 48 form an essential catalytic dyad in the fashion of serine proteases (10). Tyr 52 and Tyr 73 appear to be associated with these two residues via a hydrogen bonding network, but there is evidence (11) that Tyr 52 is not essential for the catalytic reaction.

From structure-function studies of Type I and II enzymes, the first eight residue at the N-terminus together with Tyr 69, based on the

Renetseder et al numbering system (see Figure 11)(12,12a), are thought to play a functional role in interfacial binding of the enzyme to aggregated substrate (13). Both chemical modification (14) and site-directed mutagenesis (15-17) studies have shown that the N-terminal residue is
5 crucial for activity. Further studies with Type I PLA2 (17, 18) have shown that significant alterations of the invariant hydrophobic face of the N-terminal amphipathic helix (residues 2, 5 or 9) are detrimental to enzyme activity. Residue 10 is necessary for interfacial binding and changes in other residues have modest or no effect on catalytic activity. Production of the
10 fully active enzyme depends on conformational changes of the N-terminal helical region which occur, firstly, on interfacial binding to the phospholipid micelle and secondly on substrate binding at the active site (19).

NMR studies of porcine pancreatic PLA2 indicated that the first few residues of the N-terminus are not α -helical in solution, but become helical
15 and rigid on formation of a ternary complex with micelles or a substrate analogue (20-22). X-ray crystallographic studies showed that the N-terminal and b-loop region (residues 62-73) are held by hydrogen bonding in the active enzyme conformation and form the catalytic network involving His 48, Tyr 52, Tyr 73 and Asp 99 (23-26).

High affinity inhibitors of the human Type II PLA2 have been identified which bind either reversibly or irreversibly. Reversible competitive inhibitors of PLA2 have been derived from phospholipid analogues. These substrate analogues interact with the catalytic residues in the enzyme's active site thereby perturbing substrate binding. These
25 inhibitors have been designed to emulate the putative tetrahedral intermediate that forms during hydrolysis of the substrate (24,27,28) or are based on non-hydrolyzable phospholipid analogues (29,30). Schevitz et al (31) have generated a potent and selective inhibitor of human type II PLA2 which binds at the active site of the enzyme. The initial lead compound was
30 identified by large scale screening of library compounds and then further improved by minimising the interactions of other substrate analogues in the active site.

Manoalide, a natural product derived from sponge, and its analogues have been demonstrated to possess anti-inflammatory properties associated
35 with inhibition of PLA2 (32). These compounds inhibit PLA2 by a mechanism that does not directly involve the catalytic site. The inhibitory

reaction is irreversible and involves covalent modification of specific lysine residue of the enzyme (33).

Recently, Type II PLA2 has also been shown to upregulate prostaglandin biosynthetic pathways independently of its enzyme activity (43). This implies that the enzyme may function by binding to cell surface receptors and modulating intracellular signalling pathways. It is possible that this mechanism may involve amino acid residues in the vicinity of the active site channel.

There are a number of cases where knowledge of protein structure has contributed to the design of new therapeutic agents and/or an understanding of the action of existing agents. Examples of these include influenza virus neuraminidase (34), HIV protease (for a selected sample see reference 35), purine nucleoside phosphorylase (36) and thymidylate synthase (37). The use of peptides as lead structures in the development of non-peptidic drugs is therefore achievable.

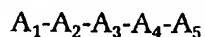
Modern computational technologies have improved these drug development strategies. Design strategies for peptide leads have been previously described (38). In order to understand how an enzyme binds an inhibitor, automated docking methods are invaluable where there is a knowledge of the enzyme structure from X-ray crystallographic (39) or nuclear magnetic resonance methods (40). The interactions are fundamental to molecules and can be described by energy terms. These methods, or variations thereof, in the drug design process are generally referred to as "structure-based drug design" (41). Many more methods become viable as computational technology grows.

The present inventors have previously identified a class of compounds which inhibit the enzyme activity of Type II phospholipase A2 (WO 93/01215). It was postulated that this inhibition is mediated by the peptide binding to the amino terminal residues of the enzyme and blocking the reaction either by blocking substrate access to the hydrophobic channel or by distorting the structure sufficiently to prevent correct orientation of the substrate.

Summary of the Invention

The present inventors have now identified specific cyclic peptide inhibitors of PLA2 which demonstrate unexpectedly high inhibitory activity.

Accordingly, in a first aspect, the present invention provides a cyclic peptide which inhibits the enzymatic activity of Type II PLA2, the peptide having the following formula:



5 in which

A_1 is F or Y or W or 2Nap

A_2 is L or I

A_3 is S or T

A_4 is F or Y or W or 2Nap

10 A_5 is R or K.

In a preferred embodiment of the first aspect the present invention, the peptide is selected from the group consisting of cFLSYK, cFLSYR and c(2Nap)LS(2Nap)R.

15 When used herein the term "cFLSYK" means "cyclic FLSYK", "cFLSYR" means "cyclic FLSYR" and "c(2Nap)LS(2Nap)" means "cyclic (2Nap)LS(2Nap)". The term "2Nap" is an abbreviation for 2-naphthylalanine.

In a further preferred embodiment, the peptide comprises D-amino acids and has a sequence which corresponds to the reverse sequence of a peptide according to the first aspect of the present invention.

20 In a second aspect the present invention provides a composition for use in treating a subject suffering from septic shock, rheumatoid arthritis and/or other inflammatory diseases, the composition including a therapeutically acceptable amount of a peptide according to the first aspect of the present invention and a pharmaceutically acceptable carrier.

25 In a third aspect the present invention provides a method of treating septic shock and/or inflammatory disease in a subject which includes administering to the subject a peptide of the first aspect of the present invention.

30 It will be appreciated by those skilled in the art that a number of modifications or substitutions may be made to the cyclic peptides of the present without substantially decreasing the biological activity of the peptide. This may be achieved by various changes, such as insertions, deletions and substitutions, either conservative or non-conservative in the peptide sequence where such changes do not substantially decrease the biological activity of the peptide. By conservative substitutions of the side

35

chains the intended combinations may embrace polarity (n,q,s,t,y;d,e;k,r,h), hydrophobicity (v,i,l,m,f,w,y,k) and aromaticity (f,y,w).

It may also be possible to add various groups (which include organic molecules and peptidomimetics) to the peptides of the present invention to confer advantages such as increased potency without substantially decreasing the biological activity.

Modifications of the peptides contemplated herein include, but are not limited to, modifications to side chains, incorporation of unnatural amino acids and/or their derivatives during peptide synthesis and the use of crosslinkers and other methods which impose conformational constraints on the peptides.

Those skilled in the art will appreciate that other peptide and non-peptide compounds which provide substantially the same spatial geometry and polarity as the cyclic peptides of the present invention should also mimic the inhibitory effects of the cyclic peptides of the present invention. It is intended that such peptides and compounds are also included within the scope of the present invention.

Accordingly, the present invention extends to a compound wherein the spatial geometry and polarity of the compound substantially corresponds to that of a peptide according to the first aspect of the present invention, wherein the compound is capable of inhibiting the enzymatic activity of Type II phospholipase A2.

It will be also appreciated by those skilled in the art that the peptides of the present invention provide model structures which may be used to design or screen for compounds (either naturally occurring or synthetic) which have PLA2 inhibitory activity.

Accordingly, the present invention extends to a method of screening an agent for potential PLA2 inhibitory activity which method includes analysing the structure of the agent for similarities with the three dimensional structure of a peptide according to the first aspect of the present invention. The present invention also extends to a method of generating agents with PLA2 inhibitory activity which includes generating agents which share similarities with the three dimensional structure of a peptide according to the first aspect of the present invention.

Throughout this specification, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a

stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

It will be also appreciated by those skilled in the art that the peptides of the present invention also provide model structures which may be used to identify specific residues on the PLA₂ enzyme which are involved in inhibition of the enzyme.

Brief Description of the Figures

- 10 **Figure 1a.** Inhibition of Type IIa secretory PLA₂ by Cyclic Peptide FLSYR. PLA₂ assay was performed using labelled *E.coli* membranes as described in Tseng et al. (1996) J. Biol. Chem. 271(39):23992.
- Figure 1b.** Inhibition of Type IIa secretory PLA₂ by Cyclic Peptide (2-nap)LS(2-nap)R.
- 15 PLA₂ assay was performed using labelled *E.coli* membranes as described in Tseng et al. JBC 1996.
- Figure 2.** Effect of Cyclic Antagonists on sPLA₂-mediated PGE₂ Release from Synovial Fibroblasts.

Detailed Description of the Invention

The peptides of the present invention may be synthesised using techniques well known to those skilled in this field. For example, the peptides may be synthesised using solution synthesis or solid phase synthesis as described, for example, in Chapter 9 entitled "Peptide Synthesis" by Atherton and Sheppard which is included in a publication entitled "Synthetic Vaccines" edited by Nicholson and published by Blackwell Scientific Publications, the entire contents of which are incorporated herein by reference.

Cyclisation may be achieved as follows. The peptide may be assembled using standard f-moc solid phase chemistry on an acid-labile resin. The fully-protected peptide may be cleaved from the resin and cyclised by formation of an amide bond using standard peptide synthesis activation and coupling chemistry. The cyclic peptide may be deprotected, purified using reverse phase HPLC and the purity and identity confirmed by mass spectrometry.

The peptides of the present invention provide model structures which may be used to design or screen for compounds (either naturally occurring or synthetic) which have PLA2 inhibitory activity, and which may be used to identify specific residues on the PLA2 enzyme which are involved in inhibition of the enzyme. These model structures will allow the design of compounds or agents which bind efficiently to the same site as the peptides of the present invention. The techniques involved in these approaches, such as x-ray crystallographic techniques and computer based modelling, are now well advanced.

In general, the design of a molecule possessing stereochemical complementarity can be accomplished by means of techniques that optimize, either chemically or geometrically, the "fit" between a molecule and a target receptor. Known techniques of this sort are reviewed by Sheridan and Venkataraghavan, *Acc. Chem. Res.* 1987 20 322; Goodford, *J. Med. Chem.* 1984 27 557; Beddell, *Chem. Soc. Reviews* 1985, 279; Hol, *Angew. Chem.* 1986 25 767 and Verlinde C.L.M.J & Hol, *W.G.J. Structure* 1994, 2, 577, the respective contents of which are hereby incorporated by reference. See also Blundell et al., *Nature* 1987 326 347 (drug development based on information regarding receptor structure).

The geometric approach is illustrated by Kuntz et al., *J. Mol. Biol.* 1982 161 269, the contents of which are hereby incorporated by reference, whose algorithm for ligand design is implemented in a commercial software package distributed by the Regents of the University of California and further described in a document, provided by the distributor, which is entitled "Overview of the DOCK Package, Version 1.0," the contents of which are hereby incorporated by reference. Pursuant to the Kuntz algorithm, the shape of the cyclic peptide is defined as a series of overlapping spheres of different radii. One or more extant data bases of crystallographic data, such as the Cambridge Structural Database System maintained by Cambridge University (University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, U.K.) and the Protein Data Bank maintained by Brookhaven National Laboratory (Chemistry Dept. Upton, NY 11973, U.S.A.), is then searched for molecules which approximate the shape thus defined.

Molecules identified in this way, on the basis of geometric parameters, can then be modified to satisfy criteria associated with chemical

complementarity, such as hydrogen bonding, ionic interactions and Van der Waals interactions.

The chemical-probe approach to ligand design is described, for example, by Goodford, J. Med. Chem. 1985 28 849, the contents of which are hereby incorporated by reference, and is implemented in several commercial software packages, such as GRID (product of Molecular Discovery Ltd., West Way House, Elms Parade, Oxford OX2 9LL, U.K.). pursuant to this approach, the chemical prerequisites for a site-complementing molecule are identified at the outset, by probing the active site (as represented via the atomic coordinates shown in Fig. 1) with different chemical probes, e.g., water, a methyl group, an amine nitrogen, a carboxyl oxygen, and a hydroxyl. Favoured sites for interaction between the active site and each probe are thus determined, and from the resulting three-dimensional pattern of such sites a putative complementary molecule can be generated.

In order that the nature of the present invention may be more clearly understood, preferred forms thereof will now be described with reference to the following examples.

Examples

Inhibition of PLA2 activity by the cyclic peptides of the present invention was measured using labelled *E.coli* membranes as described in Tseng et al (1996) J. Biol. Chem. 271(39):23992. Inhibition by FLSYR is shown in Figure 1a and inhibition by c(2Nap)LS(2Nap)R is shown in Figure 1b.

These results show that cFLSYR (and cFLSYK, data not shown) inhibit human Type IIa PLA2 in the *E.coli* assay approximately 5-10 fold greater potency than linear FLSYK (Figure 1a). The compound c(2-nap)LS(2-nap)R is 50-100 fold more potent than linear FLSYK in this assay (Figure 1b).

The effect of cyclic antagonists on sPLA2-mediated PGE2 release from synovial fibroblasts was measured as follows. Rheumatoid synovial fibroblast cells were grown to confluence in 24 well plates. Cells were washed twice with PBS and incubated for 24 hours in Hams/DMEM containing 0.1% BSA and TNF- α (10ng/ml) in the presence or absence of sPLA2 (1ug/ml) with varying doses of either cFLSYR or c(2-nap)LS(2-nap)R. Supernatants were harvested and assayed for PGE2 using an enzyme immunoassay (Cayman Chemical). Experiments were performed in triplicate and data shown in the mean +/- SE of triplicate determination from one

representative experiment. The results of these experiments are shown in Figure 2.

The results shown in Figure 2 establish the following:

- (i) At all tested concentrations of cyclic inhibitor, TNF- α -stimulated PGE₂ release is constant at approximately 1000pg/ml. This indicates that the compounds have no effect on the TNF- α response of these cells at any dose. These data coupled with the toxicity data (see below) show that the compounds do not apparently interfere with normal cell function.
- (ii) Type IIa sPLA₂ (1ug/ml) in the absence of added inhibitor, induces a 2.5- fold augmentation of cytokine-stimulated PGE₂ release.
- (iii) At all tested concentrations, both cyclic inhibitors significantly reduce sPLA₂- stimulated PGE₂ release. cFLSYR complete abrogates this response of 1 μ M and has an IC50 below 0.1 μ M in this assay. Since sPLA₂ is present in this assay at 0.07 micromolar, this inhibition approaches a 1:1 molar ratio of enzyme to inhibitor indicating potent inhibition.

The effect of the cyclic peptides of the present invention on viability of synovial fibroblasts in culture was measured as follows. Human synovial fibroblast cells, isolated from a patient with rheumatoid arthritis, were grown to confluence in Hams/DMEM medium containing 10% FBS in 24 well plates. Cells were washed twice with PBS prior to treatment. Treatments were added to cells in Hams/DMEM containing 0.1%BSA and incubated for 24 hours at 37°C. Cells were detached from the wells with trypsin/EDTA and counted in a haemocytometer. Non-viable cells were identified by staining with trypan blue. Results are shown in Table 1.

Table 1. Effect of Cyclic Peptides on Viability of Synovial Fibroblasts in Culture.

| Treatment | Total Cell Count (Cells/well +/- SD $\times 10^{-5}$) | Non-Viable Cells (Cells/well +/- SD $\times 10^{-5}$) | % Viable Cells (+/- SD) |
|--------------------------------------|--|--|----------------------------|
| cFLSYR | | | |
| 0.1uM | 40.8 +/- 6.2 | 1.05 +/- 0.03 | 97.5 +/- 0.4 |
| 1.0uM | 30.0 +/- 2.6 | 0.60 +/- 0.02 | 98.0 +/- 0.9 |
| 10.0uM | 28.0 +/- 2.9 | 0.22 +/- 0.03 | 99.2 +/- 1.0 |
| 100.0uM | 38.1 +/- 4.3 | 0.69 +/- 0.29 | 98.2 +/- 0.7 |
| c2napLS2napR | | | |
| 0.1uM | 33.3 +/- 9.3 | 0.37 +/- 0.04 | 99.1 +/- 1.1 |
| 1.0uM | 32.7 +/- 8.1 | 0.60 +/- 0.03 | 98.3 +/- 0.7 |
| 10.0uM | 24.6 +/- 10.7 | 0.60 +/- 0.06 | 97.9 +/- 2.2 |
| 100.0uM | 3.1 +/- 1.6 | 0 | ND |
| Vehicle Alone (1.0uM DMSO) | 34.2 +/- 5.4 | 0.70 +/- 0.17 | 97.9 +/- 0.5 |

5 These data demonstrate that both cyclic compounds were non-toxic to cells at doses up to 10uM while cFLSYR was not toxic in 100uM. However, after five hours of culture, toxicity was observable in the 100uM c(2nap)LS(2nap)R-treated wells. This toxicity was confirmed by the low total cell count in this treatment (see Table I).

10 It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

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Claims:

1. A cyclic peptide which inhibits the enzymatic activity of Type II PLA₂,
5 the peptide having the following formula:
A₁-A₂-A₃-A₄-A₅
in which
A₁ is F or Y or W or 2Nap
A₂ is L or I
10 A₃ is S or T
A₄ is F or Y or W or 2Nap
A₅ is R or K.
2. A peptide as claimed in claim 1 selected from the group consisting of
15 cFLSYK, cFLSYR and c(2Nap)LS(2Nap)R.
3. A composition for use in treating a subject suffering from septic shock,
rheumatoid arthritis and/or other inflammatory diseases, the composition
comprising a therapeutically acceptable amount of a peptide according to
20 claim 1 or claim 2 and a pharmaceutically acceptable carrier.
4. A method of treating septic shock and/or inflammatory disease in a
subject which comprises administering to the subject a peptide according to
claim 1 or claim 2.
25
4. A compound wherein the spatial geometry and polarity of the
compound substantially corresponds to that of a peptide according to claim 1
or claim 2, wherein the compound is capable of inhibiting the enzymatic
activity of Type II phospholipase A₂.
30
5. A method of screening an agent for potential PLA₂ inhibitory activity
which method includes analysing the structure of the agent for similarities
with the three dimensional structure of a peptide according to claim 1 or
claim 2.
35

6. A method of designing a compound with PLA2 inhibitory activity which includes designing a compound which shares similarities with the three dimensional structure of a peptide according to claim 1 or claim 2.

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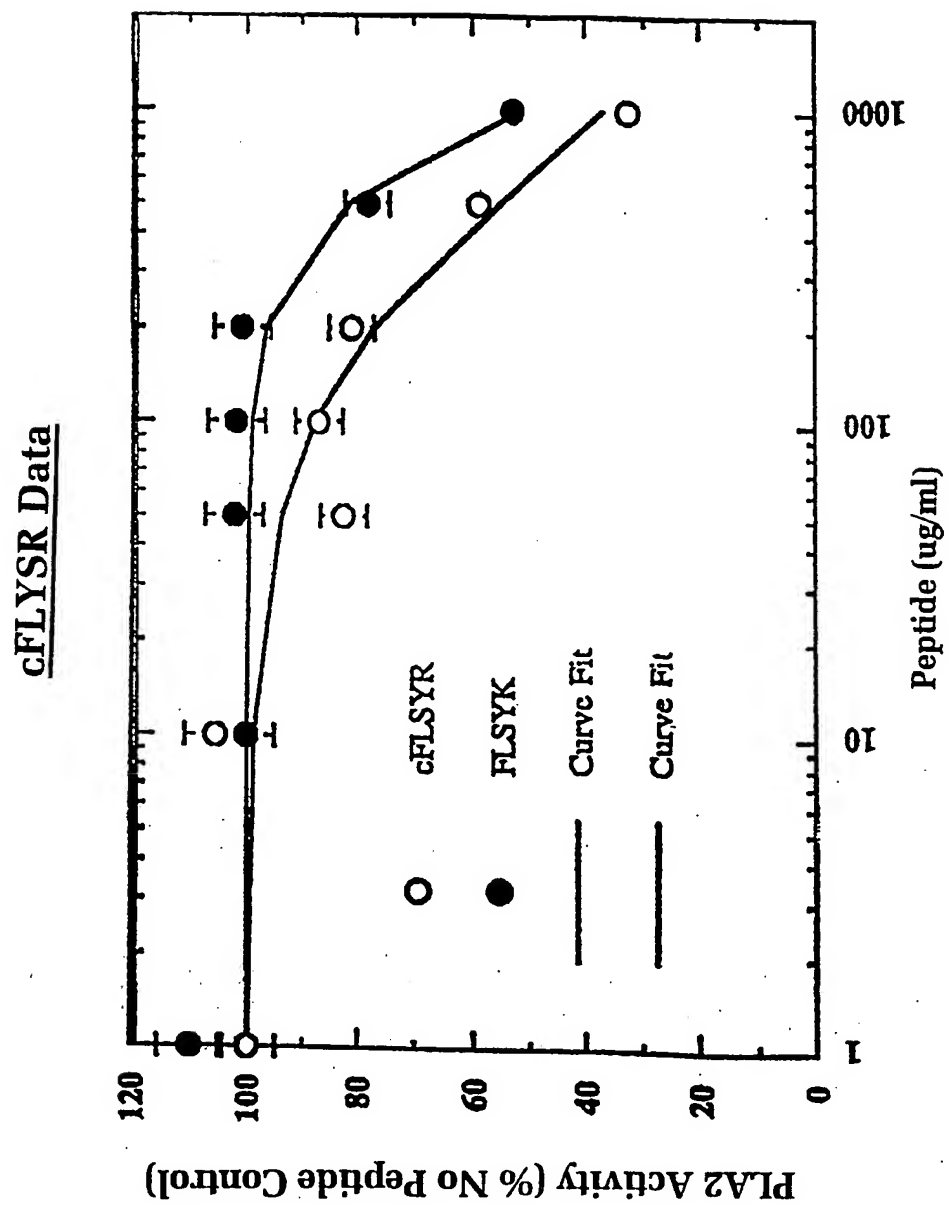


Figure 1A

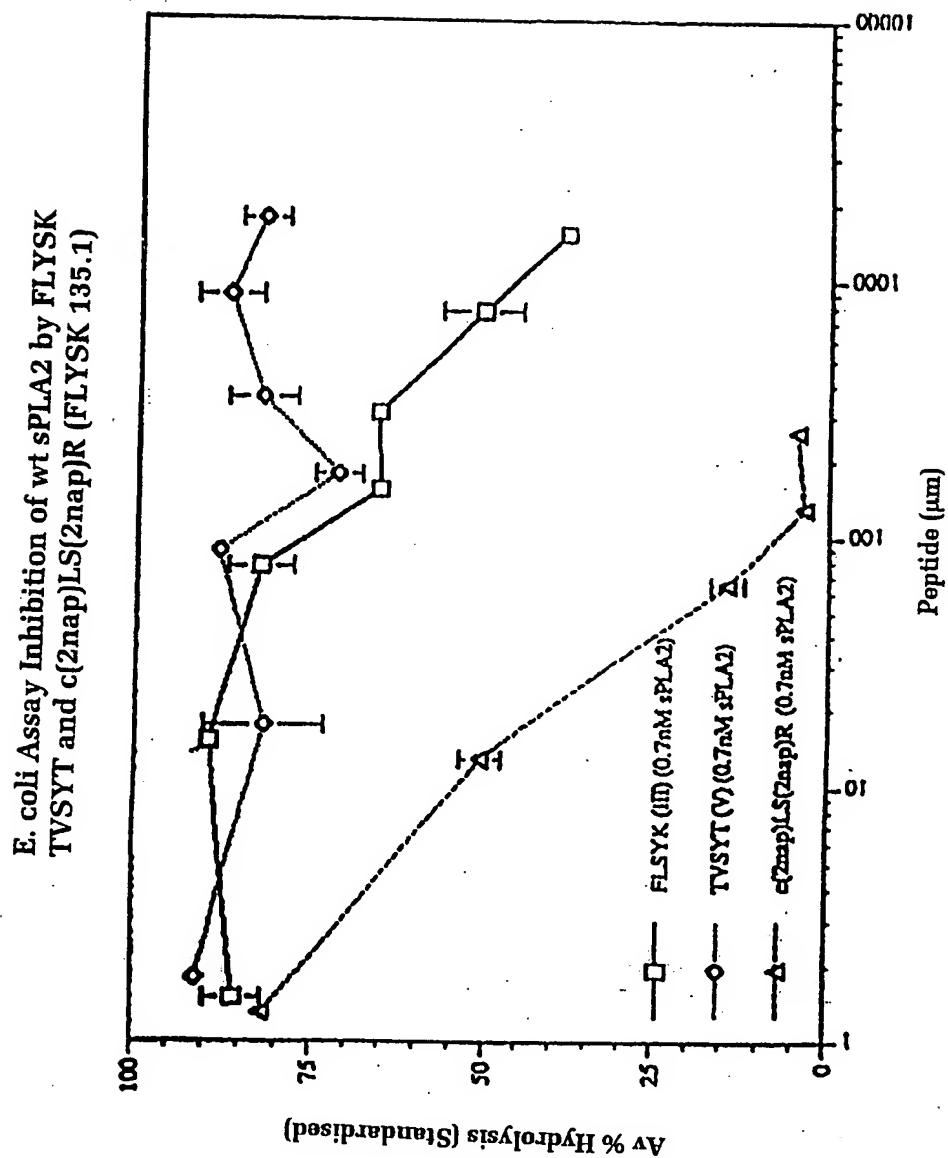


Figure 1B

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Effect of Cyclic Antagonists on sPLA₂-mediated
PGE₂ Release in Rheumatoid Synovial Fibroblasts

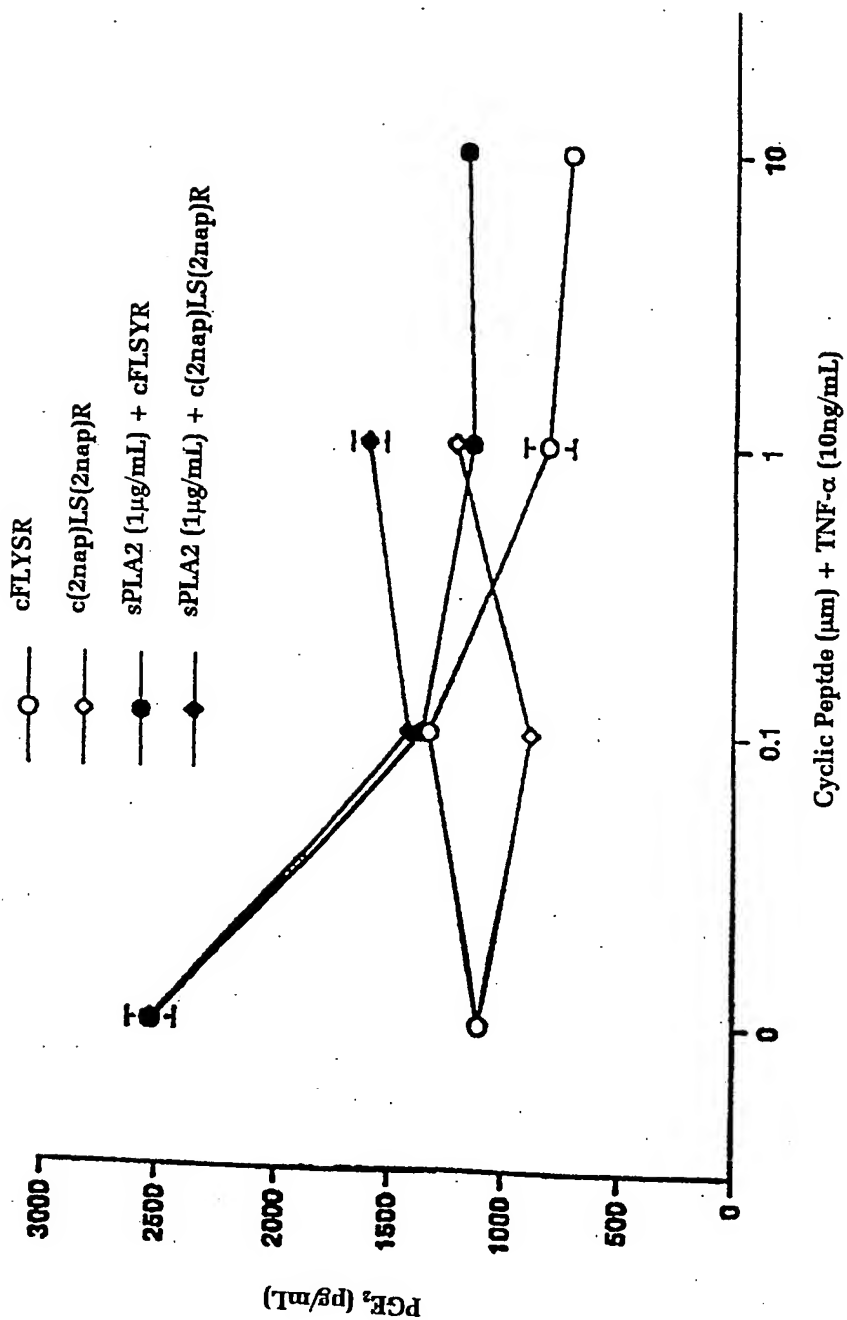


Figure 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 99/00087

| | | | | |
|--|---|---|---|---|
| A. CLASSIFICATION OF SUBJECT MATTER | | | | |
| Int Cl ⁶ : C07K 7/64 A61K 38/12 G01N 33/68 | | | | |
| According to International Patent Classification (IPC) or to both national classification and IPC | | | | |
| B. FIELDS SEARCHED | | | | |
| Minimum documentation searched (classification system followed by classification symbols) AU:IPC as above | | | | |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched C07K 7/06 | | | | |
| Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) STN: [LI], [ST], [RK], SQL=5, cyclic | | | | |
| C. DOCUMENTS CONSIDERED TO BE RELEVANT | | | | |
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. | | |
| X | AU 22732/92-B(668513) (21 January 1993) to Garvan Institute of Medical Research, et al - See whole document, especially pages 3 and 6, claims 1 to 12 and 18 to 19. | 1, 2, 3, 4 | | |
| P, X | AU 43712/97 A -(2 April 1998) to Garvan Institute of Medical research, et al. See whole document especially pages 3 to 12 | 1-6 | | |
| A | Märki, Fritz et al "Recombinant Human Synovial Fluid Phospholipase A2..." J. Biochem, 113, pp 734-737 (1993). | | | |
| <input type="checkbox"/> Further documents are listed in the continuation of Box C <input checked="" type="checkbox"/> See patent family annex | | | | |
| <p>* Special categories of cited documents:</p> <table style="width: 100%;"> <tr> <td style="width: 50%; vertical-align: top;"> <p>"A" Document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </td> <td style="width: 50%; vertical-align: top;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p> </td> </tr> </table> | | | <p>"A" Document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> | <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p> |
| <p>"A" Document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> | <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p> | | | |
| Date of the actual completion of the international search 3 March 1999 | | Date of mailing of the international search report - 9 MAR 1999 | | |
| Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (02) 6285 3929 | | Authorized officer K.G. ENGLAND Telephone No.: (02) 6283 13 February 1998 | | |

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU 99/00087

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent Document Cited in Search Report | | | | Patent Family Member | | | |
|--|----------|----|---------|----------------------|---------|-------|---------|
| AU | 22732/92 | EP | 592553 | US | 5222134 | CA | 2095723 |
| | | EP | 556305 | WO | 9301215 | WO | 9209160 |
| | | US | 5103476 | AT | 171024 | JP B4 | 7089345 |
| END OF ANNEX | | | | | | | |